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Flavonoids from Leaves and Exocarps of the Grape Kyoho

Hye-Jeong Park and Hyeon-Cheol Cha*

Department of Biology, College of Advanced Sciences, Dankoook University, Cheonan 330-714, Korea

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We analyzed and compared profiles of flavonols extracted from leaves and exocarps of the grape Kyoho by TLC, HPLC and UV spectrophotometry. In the exocarps, quercetin 3-O-glucoside was the main compound while isorhamnetin 3-O-glycoside (I) was present in minor amounts. In leaves, on the other hand, quercetin 3-O-glucoside and quercetin 3-O-glucoside-7-O-glucronide were the major compounds while isorhamnetin 3-O-glycoside (II) and kaempferol 3, 7-O-diglycoside were present in minor amounts.

Flavonoids are 15-carbon phenolic compounds generally distributed throughout the plant kingdom (Harbone, 1984) and divided into isoflavones, anthocyanidins, flavans, flavonols, flavones and flavonones (Peterson and Dwyer, 1998).

Among them, flavonols play important roles in the protection of plants from UV damage, as well as regulation of stem elongation, dormancy and fruit maturation. It is also known that grape flavonols affect the co-pigmentation with anthocyanins (Asen et al., 1972). The phenolic compounds are important constituents of plant cells and are associated with physiological defense against infection by bacteria and viruses. Moreover, the phenolic compounds in grape and wines reportedly contribute to the followoing properties: color, astringency, bitterness, oxidation reactions, interaction with proteins and aging behavior of wine (Carando et al., 1999). Recently, the flavonoid compounds have received considerable attention due to their antioxidant, antimutagenic, and anticarcinogenic properties (Karakaya and El, 1999; Soleas et al., 2002). Although they are not considered essential nutrients, some flavonoids, such as quercetin and rutin, are known to support human health by serving as anti-inflammatory, antihistaminic, and antiviral agents (Soleas et al., 1997).

Kyoho grape is very popular among Korean consumers. Although the flavonoid components in *Vitis vinifera* L. have been extensively studied (Garcia-Viguera and Bridle, 1995; Betes-Saura et al., 1996; Van Wiel et al., 2001; Tsanova-Savova and Ribarora, 2002), those of tetraploid grape such as *Vitis labruscana* cv. Kyoho are unknown. We analyzed flavonol profiles in the leaves and exocarps of 'Kyoho' and compared them by TLC, HPLC and UV spectra. This paper presents flavonol profiles of leaves and exocarps of Kyoho grape.

*To whom correspondence should be addressed.
Tel: 82-41-550-3444, Fax: 82-41-551-9229
E-mail: hccha@dankook.ac.kr

Materials and Methods

Reagents

Standards of rutin, quercetin and sugars for analysis were purchased from Sigma (St. Louis, MO, USA). All solvents used for extraction and HPLC analysis were obtained from Fisher Scientific (Santa Clara, CA, USA) and Merck (Darmstadt, Germany).

Plant materials

Analysis of flavonoid profiles was performed on the leaves and exocarps of the grape Kyoho. They were collected from the local vineyard on August 20, 2002, when most of fruits were ripening. They were immediately freeze-dried and were stored at -60°C for future analysis.

Preparation of the crude flavonols extracts

Twenty grams of freeze-dried leaves and exocarps were ground at a high speed with blender and extracted overnight in 85% MeOH at room temperature. The slurry was filtered through Whatman No. 1 filter paper in a Büchner funnel and the residue was re-extracted overnight in 50% MeOH at room temperature. The MeOH extract was evaporated under reduced pressure and the residue was dissolved in H_2O. This crude extract was dissolved in chloroform to separate flavonoids from chlorophyll and then the residual aqueous phase was washed with ethyl acetate. The extracts were evaporated and stored at -4°C.

Analysis of flavonoids

The composition of flavonoids was analyzed by HPLC, using Gilson Model 305-306/115 UV with dual pump

system (Villiers le Bel, France). The column was Waters semi-preparative μ-Bondapak C-18 column (Waters, Milford, MA, USA, 7.8x30 mm I.D.) and the detection of the compounds was carried out at 254 nm.

The HPLC protocol employed was the one proposed by Mun and Park (1995). The mobile phase consisted of acetonitrile for pump A and 2% aqueous acetic acid solution for pump B, and the gradient elution was carried out with 15-30% acetonitrile in 2% acetic acid solution for 27 min after start of the program. Flow rate was maintained at 3 ml/min. All solvents were of HPLC-grade and were filtered and degassed before use.

The preliminary analysis of the extracts containing flavonoids was performed using two-dimensional TLC (Merk cellulose plate, thickness 100 μm; solvents TBA, HOAc; Table 2).

Bulk isolation and purification of the flavonoids was carried out by one-dimensional paper chromatography in 15% aqueous acetic acid solution using Whatman 3 MM paper followed by HPLC.

Purified flavonoids were identified on the basis of UV spectral analysis, acid hydrolysis, R_f values, retention times, and co-chromatography with standard using HPLC and TLC according to the method described by Harbone (1984) and Markham (1982).

Results and Discussion

The flavonols contained in the exocarps and leaves of the grape Kyoho were subjected to HPLC under the conditions described. The results of the screening test for flavonoid identification of Kyoho are shown Tables 1 and 2.

In the exocarps of Kyoho grape, quercetin 3-O-glucoside was the main compound while isorhamnetin 3-O-glycoside (I) was present in minor amounts. In leaves, however, in quercetin 3-O-glucoside, as well as quercetin 3-O-glucoside-7-O-glucuronide, were major compounds, while isorhamnetin 3-O-glycoside (II) and kaempferol 3, 7-O-diglycoside were present in minor amounts (Tables 1, 2).

According to Van Wiel et al. (2001) and Tsanova-Savova and Ribarora (2002), the most common flavonoids in grape wine were flavonols (quercetin, kaempferol, and myricetin). Garcia-Viguera and Bridle (1995) analyzed non-colored phenolic compounds in a Portuguese red wine made from *Vitis vinifera* cv. Roriz to detect other compounds besides the above mentioned three kinds of flavonols and isorhamnetin. On the contrary, Betes-Saura et al. (1996) detected only quercetin 3-glucuronide

Table 2. Chromatographic properties of flavonoids identified from leaves and exocarps of Kyoho grape

Compound	RT		R_f value	
	(min)	α	TBA	HOAc
Quercetin 3-O-glucoside	10.84	1.04	0.48	0.29
Quercetin 3-O-glucoside-7-O-glucuronide	10.82	1.06	0.38	0.27
Isorhamnetin 3-O-glycoside (I)	12.58	1.24	0.60	0.34
Isorhamnetin 3-O-glycoside (II)	10.28	1.01	0.34	0.43
Kaempferol 3, 7-O-diglycoside	15.04	1.48	0.72	0.38

RT; absolute retention time. α; RT/RT(internal standard). Solvents; tertiary butanol : acetic acid : water (3:1:1, v/v/v), HOAc; acetic acid : water (15 : 85, v/v)

Table 1. The UV spectral data of the flavonoids in leaves and exocarps of Kyoho grape

Compound	Absorption maxima (nm)					
	MeOH	NaOMe	$AlCl_3$	AlCl/HCl	NaOAc	NaOAc/H_3BO_3
Quercetin 3-O-glucoside	351.4 265sh[1] 257.4	402.6 325.6sh 272.6	422 362.2sh 299.6sh 274.4	395.8 356.8 300.5sh 271	389.4 260.4	357 260.4
Quercetin 3-O-glucoside-7-O-glucuronide	361.0 300sh 268sh 256	408.8 332.6 273.4	426.6 365sh 303.4sh 274	398 362 300.8sh 269	371.8 291.8sh 261.8	364.4 298.8sh 260.6
Isorhamnetin 3-O-glycoside (I)	350.4 266.0	413.2 326.4sh 282.0	419.0 359.8sh 303.2sh 275.6	392.8sh 353.4 298.6sh 273.2	386.6 303.4sh 271.8	361.4 263.6
Isorhamnetin 3-O-glycoside (II)	357.8 289sh 268sh 257.4	411.8 317.8sh 272.8	405 358.4 305.4sh 272.2	393.4 358.2 303sh 269.2	362.6 301.8sh 259.6	358 264.8
Kaempferol 3, 7-O-diglycoside	343.8 300.6sh 265.6	395.6 323.2 275.8	400sh 349 303.2 268	395.5sh 343.8 299.6sh 269.6	344.2 265	343.4 266

[1]shoulder

Table 3. Distribution of flavonoid compounds identified from leaves and exocarps of Kyoho grape

Compound	Source	
	Exocarp	Leaf
Quercetin 3-O-glucoside	+++	+++
Quercetin 3-O-glucoside-7-O-glucuronide		+++
Isorhamnetin 3-O-glycoside (I)	+	
Isorhamnetin 3-O-glycoside (II)		+
Kaempferol 3, 7-O-diglycoside		+

+; Light spot, ++; Spot of average intensity, +++; Heavy spot.

in grape juice and wine. In leaves and exocarps of grape *Vitis labruscana* cv. Kyoho, we found only quercetin, kaempferol, and isorhamnetin and could not detect myricetin derivatives (Table 1 and 2). These different results suggest that different kinds of flavonols are present in different grape species and cultivars.

It was also known that the profiles of flavonols were different among specific organs or processing methods even in the same grape. For example, Lu and Foo (1999) identified a variety of flavonoids (quercetin 3-O-glucoside and 3-O-glucuronide, kaempferol 3-O-glucoside and 3-O-galactoside, eucryphin, astilbin and eugeletin) in grape pomace. Justesen et al. (1998) also found quercetin, keampferol, hesperetin and naringenin in the pulp of grape fruit. The quantities of flavonoids also affected by the specific organs. The content of quercetin was higher in the peels than in the grape berries and pulp (Palomino et al., 2000). Also, Negro et al. (2003) reported that the concentration of total flavonoids contained in the grape seed extract was higher than that obtained from the peel and the marc. There are other reports that important influencing factors on flavonol compositions of grapes are the viticultural area, the vintage, the time at which grapes are picked, and the storing time of the wine bottles (Andrade et al., 2001; Sellappan et al., 2002; Netzel et al., 2003). Furthermore, within the same fruit type, the growing season, variety, environmental and climatic conditions, plant disease, soil type, geographic locations, and even maturity seem to influence the concentration of phenolic compounds. We also found that the composition of flavonols found in Kyoho were different from organs to organs. In the exocarps, quercetin 3-O-glucoside and isorhamnetin 3-O-glycoside were present, whereas quercetin 3-O-glucoside, quercetin 3-O-glucoside-7-O-glucuronide, isorhamnetin 3-O-glycoside (II) and kaempferol 3, 7-O-diglycoside were present in the leaves. This finding is in agreement with the reports (Justesen et al., 1998; Palomino et al., 2000; Negro et al., 2003) that the kinds of phenolic compounds including flavonoids vary in different organs within an individual of grapes.

This study provides basic information on flavonoid composition in leaves and exocarps of *Vitis labruscana* cv. Kyoho. The kinds of flavonols in the exocarps were less than those in leaves. From this observation, we speculate that stored flavonols in fruit skin might be associated with the biosynthesis of anthocyanin pigments.

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